THE USE OF THE ACTIVATED N-TERMINAL SIXTEEN AMINO ACID PEPTIDE OF THE ANTINEOPLASTIC PROTEIN (ANUP) AS A PHARMACOLOGICALLY ACTIVE ANTI-TUMOR AGENT

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~~References Cited~~

~~U.S. Patent Documents~~

~~4,359,415 11/1982 Sloane~~
~~4,559,325 12/1985 Burzynski~~
~~5,008,372 4/1991 Wellner~~
~~5,928604 3/1994 Sloane~~

~~U.S. Application Number 08/641,905 05/02/96 Sloane~~

~~OTHER PUBLICATIONS~~

~~Sloane et al. Biochemical Journal (1986), 234, pp. 355-362.~~
~~Pottathil et al., Cancer Res. Therapy and Control (1990), 1, pp. 193-198.~~
~~Struve et al. Cancer Res. Therapy and Control (1990) 1: pp. 225-230.~~
~~Ridge and Sloane, Cytokine (1996) 8 pp. 1-5~~
~~Sloane and Davis, Tumor Targeting (1996) 2 pp 322-326.~~

~~ABSTRACT~~

~~The 16 amino acid peptide representing the partial N-terminal amino acid sequence of the Antineoplastic Protein (ANUP) is a highly active pharmacologically antitumor agent. The 16 amino acid peptide is about 50% as active as antitumor agent compared to the antitumor active as the protein (ANUP) per se when tested as a tumor killer agent (in vitro) utilizing human breast tumor cell line (MDA 231). The protein (ANUP) in the purified state also shows regression of both HeLa (human cervical tumor all line) and KB (human laryngeal cell line) implanted in nude mice (Sloane, Davis Tumor Targeting (1996) 2, pp 322-326. The nonapeptide is about 10% as active compared to the antineoplastic protein (ANUP) in the human breast tumor cell line in vitro assay system. Both peptides, the 9 amino acid peptide and the 16 amino acid peptide require presence of the detergent sodium dodecyl sulfate to activate the peptides for full pharmacological antitumor activity.~~

~~The ANUP N-terminal 16 amino acid peptide contains the following sequence (as L-Amino Acids):~~

~~1. Pyroglu~~
~~2. Leu~~
~~3. Lys~~
~~4. Cys~~
~~5. Tyr~~
~~6. Thr~~
~~7. Cys~~
~~8. Lys~~
~~9. Glu~~
~~10. Pro~~
~~11. Me~~
~~12. Thr~~
~~13. Ser~~
~~14. Ala~~
~~15. Ala~~
~~16. Cys (SEQ ID NO: 1)~~

~~The use of the N-terminal Sixteen Amino Acid Peptide as a Pharmacologically Active Anti-tumor Agent.~~

~~BACKGROUND OF THE INVENTION~~

FIELD OF THE INVENTION

The present invention relates to the use of the 16 amino acid peptide which represents the partial N-terminal amino acid sequence of the Antineoplastic Protein (ANUP) as a pharmacologically active antitumor agent. ~~The peptide is about 50% as active as the protein per se but only about one-tenth of the weight of the peptide is equivalent in activity of the protein (ANUP) on a molar basis (ca 10^{-9} M).~~

BACKGROUND OF THE INVENTION

The Antineoplastic Protein (ANUP) kills tumor cells. The protein (ANUP) in the purified state has been implicated in regression of both HeLa (human cervical tumor all line) and KB (human laryngeal cell line) implanted in nude mice.

SUMMARY OF THE INVENTION

The present invention describes the pharmacologically active anti-tumor activity of the 16 amino acid peptide which represents the partial N-terminal amino acid sequence of the Antineoplastic Protein (ANUP).

The 16 amino acid peptide is approximately one-half as active as the protein on a molar basis utilizing the human breast tumor cell line (MDA 231). However, only about one-tenth of the weight of the peptide is required when compared to the amount of protein for equivalent activity against the human breast tumor cell line. Both the protein and the peptide exert their action by killing tumor cells (apoptosis) since electron microscopy studies showed complete degradation of the cells (Struve et al. Cancer Res. Therapy and Control (1990) 1: pp 225-230).

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to the use of the 16 amino acid peptide which represents the partial N-terminal amino acid sequence of the Antineoplastic Protein (ANUP) as a pharmacologically active antitumor agent. The peptide is about 50% as active as the protein per se but only about one-tenth of the weight of the peptide is equivalent in activity of the protein (ANUP) on a molar basis (ca 10^{-9} M).

DESCRIPTION OF THE PREFERRED EMBODIMENT

The 16 Amino Acid Peptide

The synthetic hexadeca peptide (16 L-amino acids) has the following sequence:

1.	Pyroglu		9.	Glu	E
2.	Leu	L	10.	Pro	P
3.	Lys	K	11.	Met	M
4.	Cys	C	12.	Thr	T
5.	Tyr	Y	13.	Ser	S
6.	Thr	T	14.	Ala	A
7.	Cys	C	15.	Ala	A
8.	Lys	K	16.	Cys	C (SEQ ID NO: 1)

The peptide was synthesized by Research Genetics Inc., in Huntsville, AL 35801; the peptide was pure as shown by HPLC (high performance liquid chromatography) and the molecular weight was check by mass spectrometry (MS).

The 16 amino acid peptide representing the partial N-terminal amino acid sequence of the Antineoplastic Protein (ANUP) is a highly active pharmacologically antitumor agent. The 16 amino acid peptide is about 50% as active as antitumor agent compared to the antitumor active as the protein (ANUP) per se when tested as a tumor killer agent (in vitro) utilizing human breast tumor cell line (MDA 231). The protein (ANUP) in the purified state also shows regression of both HeLa (human cervical tumor all line) and KB (human laryngeal cell line) implanted in nude mice (Sloane, Davis Tumor Targeting (1996) 2, pp 322-326). The nonapeptide is about 10% as active compared to the antineoplastic protein (ANUP) in the human breast tumor cell line in vitro assay system. Both peptides, the 9 amino acid peptide and the 16 amino acid peptide require presence of the detergent sodium dodecyl sulfate to activate the peptides for full pharmacological antitumor activity.

EXAMPLES

Example 1: The pharmacological anti-tumor activity of the 16 amino acid peptide (P_{16})

The antitumor activity of the peptide (P_{16}) was assayed against the human breast tumor cell line (MDA 231) and its activity was compare to the in vitro antitumor effect of the "pure" protein (ANUP).

The assay for the pharmacological antitumor activities were performed as follows utilizing 96 well plates --

20,300 - 30,000 human breast tumor cells in L-15 medium (200 ~~ul~~ µl) containing 2.5 % fetal calf serum and 100 ~~ug~~ µg ~~gestamycin~~ gentamycin per ml (complete medium) were incubated at 37° in air for 120 hours; after this incubation period 50 ~~ul~~ µl of serially diluted P_{16} and ANUP were added to each well. The serial dilutions were prepared as follows: 2 mg each (the P_{16} and ANUP) were dissolved in 2 ml of complete medium containing 0.5% sodium dodecyl sulfate (SDS). The solutions were diluted in complete medium containing 0.05% SDS to a concentration of 350 ~~ug~~ µg per ml.

Dilution plates were prepared as follows:

100 ~~ul~~ µl of complete medium were added to each well and 50 ~~ul~~ µl of diluted P_{16} and ANUP were added to each well in row A thus 1.3 dilution was accomplished; 50 ~~ul~~ µl were serially diluted in the 100 ~~ul~~ µl of medium in rows B through H. Thus the range of concentrations were from 6 ~~ug~~ µg to 2 mg when 50 ~~ul~~ µl each dilution series were added to the 200 ~~ul~~ µl of the complete medium containing the MDA cells. The plates were incubated for an additional 96-120 hours. The medium was poured off and after a 90-minute incubation with 50 ~~ul~~ µl neutral red dye (0.5 ml neutral red (0.25% ethanol (0.6 ml) diluted 5.5 saline - 0.16 mm HCl) the cells were washed twice with PBS (phosphate buffer saline) at room temperature. The concentration of living cells (since only living cells absorb the dye) was determined after adding 100 ~~ul~~ µl lysing buffer (50% ethanol in 0.05 m NaH_2PO_4) the concentration of neutral red released in each well was determined using a Dynetech plate reader set at 550 mm. A unit of activity was defined as the concentration of ANUP and P_{16} for 50% killing.

Under these assay conditions the 50% end points were as follows:

ANUP 0.1 ~~ug~~ µg /well = 1.25×10^{-8} M

P_{16} 0.0 ~~ug~~ µg /well = 2.2×10^{-8} M

Thus, P_{16} is about 50% as active as ANUP on a molar basis; whereas on a weight basis only one tenth of the peptide weight is equal in activity 10 times the weight of the protein (ANUP).

In the absence of SDS neither the peptide nor the protein showed any antitumor activity. Thus the detergent is probably necessary to form the correct geometrical shape for activity as described by Sloane and Davis Tumor Targeting (1996) 2, 322-326. The data utilizing P_{16} as an antitumor agent against the human breast tumor cell line (MDA 231) are as follows:

	Fraction of the Activity relative to ANUP
P_{16} no SDS	± no Activity
P_{16} + 0.005% SDS	0.04
P_{16} + 0.02% SDS	0.50
P_{16} + 0.05% SDS	0.50

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ABSTRACT

The invention provides a 16 amino acid peptide representing the partial N-terminal amino acid sequence of the Antineoplastic Protein (ANUP). The 16 amino acid peptide provided is a highly active pharmacologically antitumor agent.

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